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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Samuel J. Landry

Art Unit: 1644

Serial No.: 09/463,590

Examiner: A. DeCloux

Filed: April 20, 2000

Customer No.: 21559

Title: PREDICTION, DETECTION, AND DESIGN OF T CELL EPITOPES

Assistant Commissioner For Patents
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. SAMUEL LANDRY

1. I am the inventor in the above-referenced patent application.
2. The invention claimed in the above-referenced application features a method for stimulating an immune response toward a naturally occurring protein by administering an altered protein containing an unstable polypeptide segment. An unstable polypeptide is defined as one that has an average hydrophobicity value that is lower than the average

hydrophobicity value of the altered protein; has a sequence conservation that is lower than a sequence conservation of the altered protein; has an amide protection factor that is lower than 10^4 wherein the altered protein is in a native conformational state; has an average amide protection factor that is lower than the average amide protection factor for the altered protein in a denatured conformational state; has an NMR order parameter (S^2) of less than 0.8; or has an average B-factor value that is higher than the average B-factor value of the altered protein. I have discovered that the insertion of such an unstable sequence in a protein (or protein fragment) increases the antigenicity of the C-terminal adjacent (or overlapping) sequence.

3. The Examiner has rejected claims 1-8, 10-13, and 15-19 for lack of an adequate written description and claims 1-8, 10-13, 15-19, and 58 as not being enabled for the insertion of an unstable polypeptide segment, other than the mobile loop of Hsp10. I disagree.

4. Hsp10 is a family of compounds that contain similar structural features, including a mobile loop. Mande et al. Science 1996, 271:203-207, of record, shows the aligned sequences for thirty-seven members of this family, including *Mycobacterium leprae*. Given the location of the mobile loop of Hsp10 in *M. leprae*, as disclosed in the instant specification, one skilled in the art could readily determine the sequences corresponding to a mobile loop in an Hsp10 of the other species described by Mande et

al. Landry et al. Biochemistry 1997, 36:10975-10986 (Exhibit A) confirms that the sequence described by Mande et al. for the mobile loop in humans is correct.

5. Hubbard Biochim. Biophys. Acta 1998 1382:191-206 (Exhibit B) reviews the art on the importance of structural flexibility in limited proteolysis. This reference shows "that higher order structure and not primary sequence is the main determinant of the site of initial hydrolysis." The present invention, as claimed, is directed to the insertion of unstable sequences into a protein to create locations that are preferentially cleaved by proteases. The precise sequence of an unstable sequence is relevant only in terms of its flexibility, and the instant specification teaches several criteria, which may be used for this determination. Using the present specification as a guide, one skilled in the art would understand the type of sequences necessary to practice the present invention.

6. Carmicle et al. J. Biol. Chem. 2002 277:155-160 (Exhibit C) showed that deletion of a portion of the mobile loop in T4Hsp10 reduced proteolytic sensitivity in the loop and reduced epitope immunodominance of the flanking epitope. This phenomenon is exemplified in the T4Hsp10 deletion variant, T4Hsp10d8C. The mobile loop has been reconstructed in three variants of T4Hsp10 (see Figure 1 in Exhibit D). In T4Hsp10mml, a homologous but sequence dissimilar segment from human Hsp10 replaces a portion of the T4Hsp10 mobile loop. In T4Hsp10HEL29 and T4Hsp10HEL38, two different 12 amino-acid segments of T4Hsp10 have been replaced with a 12 amino-acid segment from

hen egg lysozyme. The sequence is inserted at either position 29 (within the central portion of the mobile loop) or at position 38 (replacing the C-terminal end of the mobile loop and a small portion of the hydrophobic core). The increased flexibility of the mobile loops of T4Hsp10mm1, T4Hsp10HEL29, and T4Hsp10HEL38 relative to T4Hsp10d8C restored proteolytic sensitivity and immunodominance of the epitopes flanking the new mobile loop.

7. The inserted segments have an average hydrophobicity value (Grand Average Hydropathicity Score, Kyte et al. J. Mol. Biol. 1982, 157:105-132) lower than that of the altered protein (T4Hsp10d8C). Likewise, the amino acids deleted from the original T4Hsp10 have an average hydrophobicity that is lower than that of T4Hsp10d8C.

<u>Protein</u>	<u>Grand Average Hydropathicity Score</u>
T4Hsp10d8C	0.055
T4Hsp10mm1	-0.087 (insert only)
T4Hsp10Hel29	-0.508 (insert only)
T4Hsp10Hel38	-0.508 (insert only)
T4Hsp10	-0.383 (amino acids deleted to form T4Hsp10d8C)

8. The circular dichroism spectra of the HEL-inserted proteins indicate that the proteins contain beta-sheet secondary structure like T4Hsp10 and T4Hsp10d8C (Figure 2, Exhibit D). Thermal denaturation of the proteins shows cooperative unfolding (Figure 3, Exhibit D), and glutaraldehyde crosslinking shows the formation of higher order oligomers (including heptamers) (Figure 4, Exhibit D). These data indicate that

T4Hsp10HEL29 and T4Hsp10HEL38 have an overall native-like three-dimensional structure.

9. Proteolytic sensitivity is lowest in protein lacking the mobile loop and similarly high in T4Hsp10, T4Hsp10mml, T4Hsp10HEL29, and T4Hsp10HEL38 (Figures 5 and 6, Exhibit D). As expected, T4Hsp10, T4Hsp10mml (data not shown), T4Hsp10HEL29 and T4Hsp10HEL38 yield large products that are consistent with cleavage within the mobile loop. This result was confirmed by N-terminal sequencing of the largest fragment generated by digestion of T4Hsp10HEL29 and T4Hsp10HEL38 with proteinase K (data not shown). These results support the assertion that insertion of an irrelevant sequence having lower hydrophobicity than the protein average (an "unstable polypeptide segment") can introduce proteolytic sensitivity in that segment and therefore is likely to promote presentation and immunodominance of a flanking epitope.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date:

8/5/02

Dr. Samuel Landry